Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 1375–1379, March 1984
Biochemistry

Autodigestion of lexA and phage λ repressors

(*Escherichia coli*/SOS regulatory system/specific protease/activated recA protein/intramolecular hydrolysis)

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Communicated by Evelyn M. Witkin, November 28, 1983

ABSTRACT **Proteolytic cleavage of lexA repressor is an early step in derepression of the SOS regulatory system of *Escherichia coli*. *In vivo* and *in vitro* data have indicated a role for recA protein in this specific proteolytic reaction. I show here that, under certain conditions, specific *in vitro* cleavage of highly-purified lexA protein can take place in the absence of recA protein. This autodigestion reaction cleaved the same alanine-glycine bond as did the recA-dependent cleavage reac tion. Several lines of evidence argued that it was not due to a contaminating protease activity. Autodigestion was stimulated by alkaline pH. It occurred in the presence of EDTA but was stimulated several fold by the presence of Ca^{2+}, Co^{2+}, or Mg^{2+}. The reaction appeared to be first-order, and its rate was independent of protein concentration over a wide range, strongly suggesting that it is intramolecular. Purified phage λ repressor also broke down under similar conditions to yield products like those resulting from recA protein action. Phage λ repressor broke down at a far slower rate than did lexA, as previously observed in the recA-catalyzed *in vitro* reaction and *in vivo*. This correlation between the two types of cleavage also extended to the reactions with mutant repressor proteins; taken together with the site specificity, it suggests that autodigestion and recA-dependent cleavage follow, at least in part, a similar reaction pathway. These findings indicate that specific cleavage of lexA protein can be catalyzed by the protein itself and suggest that recA protein plays an indirect stimulatory role, perhaps as an allosteric effector, in the recA-dependent reaction, rather than acting directly as a protease. The protease active site and the recA-recognition site lie in the central or COOH-terminal portion of the lexA protein, since a tryptic fragment containing these portions of lexA protein could take part in both reactions.**

The SOS regulatory system of *Escherichia coli* is controlled in part by the interplay of two proteins—the lexA protein, which represses a set of unlinked genes during normal cell growth, and the recA protein, which is required *in vivo* for inactivation of lexA protein after treatments that derepress the system by damaging DNA or altering its metabolism (reviewed in ref. 1). lexA protein is inactivated by specific cleavage at an alanine-glycine bond near its center (2). This reaction requires recA function *in vivo*; *in vitro*, recA protein also participates in cleavage of lexA protein (2–4) and, at a slower rate, a few prophage repressors such as phage λ repressor (5, 6).

The recA-dependent cleavage reaction has two striking and related features that place it at the heart of the SOS regulatory system. First, recA protein is only conditionally active in the reaction; it must be activated by interaction with one or more effectors before it can support the cleavage reaction. Moreover, activation is a reversible process. These properties allow recA protein to act as a sensor of aberrant DNA metabolism or DNA damage, or both, triggering and sustaining the SOS response when and only when the conditions demand it. *In vitro* studies have shown that the recA-dependent cleavage reaction requires two types of cofactors, a nucleoside triphosphate and a single-stranded polynucleotide, and that these cofactors form a ternary complex with recA protein, a complex that is thought to be the activated form of recA protein (5, 6). Second, the level of cleavage activity *in vivo* varies markedly during various phases of the SOS regulatory cycle (7). It is believed, though not proven, that this variation in turn reflects the level of activated recA protein and that this level determines the state of the system: at low levels the system is repressed; at high levels it is induced; and at intermediate levels of recA activation, the SOS system is in a subinduced state in which some or all of the SOS genes are partially derepressed. Although the level of recA activation can apparently be influenced by many factors, it serves to integrate diverse aspects of DNA metabolism into a unitary response and in this way it controls the state of the SOS system (7, 8).

Abundant *in vivo* evidence points to the critical role of recA function in regulation of the SOS response and of prophage induction. However, the belief that recA protein is itself a specific protease is not supported by extensive chemical evidence of the type obtained with enzymes such as chymotrypsin. Published data have shown only that recA protein is required *in vitro* for the cleavage reactions to occur under the conditions described. This report shows that, under different *in vitro* conditions, the repressor substrates break down spontaneously in a reaction with similar site and substrate specificity to that of the recA-catalyzed reaction. These data suggest that the protease activity is inherent to the repressors themselves and that activated recA protein may play an indirect role in stimulating this reaction.

MATERIALS AND METHODS

Bacterial and Plasmid Strains. Bacterial strains, with relevant genotypes listed only, were the following: AB1157 *lexA*$^+$ *recA*$^+$ (9); JL468 = AB1157/F′*lacI*^{q} (4); JL652 = JL468/pJL59; JL785 = JL652 *del*(*recA-srl*)306::Tn*10*, made from JL652 by P1 transduction of *tet*^{r} with the donor DM1817, carrying *del*(*recA-srl*)306::Tn*10* (10). Plasmid pJL59 was an overproducing derivative of pBR322 carrying a fusion of *lexA* to the *tac* promoter (11); its construction will be described elsewhere.

Purification of lexA Protein and Fragments. lexA protein was purified from the *recA*$^+$ strain JL652 or from its *recA*$^-$ derivative JL785 by the following method or minor variations of it, which was derived from previous methods (4, 12, 13). Both strains carried the plasmid pJL59, which has a fusion of *lexA* to the *tac* promoter. Cells were grown at 37°C in 1% Bacto-tryptone/0.5% NaCl containing 1 μg of thiamine and 40 μg of ampicillin per ml to about 2×10^8 cells per ml; isopropylthiogalactoside was then added to 0.5 mM, and aeration was continued until cell growth had largely ceased. Cells were harvested by centrifugation and stored frozen at −20°C, then thawed, and suspended at about 0.15 g per ml

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of buffer A (50 mM Tris·HCl, pH 8/0.5 mM EDTA/1 mM dithiothreitol/10% sucrose) containing 200 mM NaCl. All operations were at 0–5°C except as noted. Phenylmethanesulfonyl fluoride and lysozyme were added to 1 mM and 0.025%, respectively. Aliquots were quick-frozen and thawed at 0°C, held 45 min at 0°C, heated 1 min at 37°C, chilled to 0°C, and centrifuged 45 min at 110,000 × g. The clear supernatant fluid was fraction I. Sufficient 10% polyethyleneimine·HCl (pH 7.9) was added with stirring to precipitate nucleic acids (ca. 0.2% final concentration); after 20 min, the suspension was centrifuged 10 min at 8000 × g. Ammonium sulfate (0.4 g/ml) was added with stirring to the supernatant fluid; after 20 min, the suspension was centrifuged 20 min at 16,000 × g. The pellet was suspended in buffer A containing 200 mM NaCl in which 0.4 g of ammonium sulfate per ml had been dissolved, centrifuged again, dissolved in buffer B (20 mM potassium phosphate, pH 7.0/0.1 mM EDTA/10% glycerol) containing 500 mM NaCl and 1 mM dithiothreitol, dialyzed overnight against the same buffer, passed through a phosphocellulose column equilibrated with the same buffer, and diluted 1:2.5 with buffer B containing 1 mM dithiothreitol (fraction II). This fraction was applied to a phosphocellulose column equilibrated with buffer B containing 200 mM NaCl and 1 mM dithiothreitol; the column was washed with the same buffer and then eluted with buffer B containing a linear gradient of 200–1600 mM NaCl (dithiothreitol was omitted).

lexA protein was eluted at about 400 mM NaCl (fraction III) and was passed over a column of Affi-Gel 501 (Bio-Rad) to give fraction IV, which was dialyzed 2 hr against buffer C (0.1 mM EDTA/10% glycerol) containing 50 mM potassium phosphate (pH 7.0) and applied to a hydroxyapatite column. The column was washed with the same buffer and then eluted with buffer C containing a linear gradient of 50–400 mM potassium phosphate (pH 7.0). lexA protein was eluted at about 160 mM potassium phosphate and was dialyzed against buffer D (10 mM Pipes·NaOH, pH 7.0/0.1 mM EDTA/10% glycerol/200 mM NaCl) and stored at 5°C or −70°C (fraction V). Fractions shown in lanes 1–5 of Fig. 1 were from strain JL785; fraction V from this strain was also used in lanes 6–12. Most studies were with protein isolated from JL652, but protein from both sources had the same behavior where tested.

The tryptic fragment TC1 (see Fig. 3) was produced by digestion of lexA protein in buffer D (adjusted to pH 8 by addition of Tris·HCl, pH 9) containing 10 mM $CaCl_2$ for 10 min at 37°C with trypsin (at a 400:1 weight ratio of lexA to trypsin), followed by addition of soybean trypsin inhibitor (5-fold weight excess over trypsin). To the digest was added 2 vol of buffer A at double strength and one vol of H_2O. This was applied to a DEAE-cellulose column equilibrated with buffer A containing 50 mM NaCl and eluted with buffer A containing a gradient of 50–350 mM NaCl; fragment TC1 was eluted at about 100 mM NaCl and was concentrated by precipitation with ammonium sulfate and dialyzed against buffer D. Peparations of wild-type and Ind^s phage λ repressors have been described (14) and were gifts of S. Cohen. recA protein (15) was the gift of S. West. Purified lexA protein was chromatographed on an AcA34 column (0.7 × 22 cm) at 2.5 cm/hr and 5°C in 6 M guanidine·HCl/50 mM Tris·HCl, pH 8. lexA protein was eluted midway between the void and included volumes. A partial tryptic digest was made as above. The reaction was stopped by addition of excess diisopropyl fluorophosphate, and the sample was run on AcA34 as above. Fragment TC1 was eluted later than intact protein but was only partially separated from it (data not shown). Protein in AcA34 column fractions was renatured by dialysis against buffer D. Autodigestion of the tryptic fragment from such fractions was assayed in the presence of soybean trypsin inhibitor at 5 μg/ml.

Amino Acid Sequence Determination. Samples of digested lexA protein for NH_2-terminal Edman degradation were prepared under conditions similar to those in Fig. 1, except that incubations contained 50 mM NaCl. Proteins were then dialyzed against 10% acetic acid and lyophilized. Analysis was as described in ref. 16 with the addition of 2 mg of Polybrene as carrier and was done at the Protein Structure Facility (Univ. of California at Davis) by A. Smith. Intact lexA protein gave the sequence Met-Lys-Ala-Leu, as expected from the DNA sequence and previous data (2, 17, 18); the recA-catalyzed reaction products gave this sequence and, in addition, Gly-Glu-Pro-Leu as previously seen (2). The autodigestion reaction products gave the same result, indicating that both reactions cleave the same bond. Sequence analysis of fragment TC1 gave ^{68}Leu-Leu-Gln-Glu-Glu. Because autodigestion of TC1 gave a fragment with the same gel mobility as the COOH-terminal fragment from Gly-85 to Leu-202 of lexA protein (Fig. 3), I infer that TC1 extends to the COOH-terminus of lexA.

Other Methods. $NaDodSO_4$/PAGE (13% gel) was as described (7); gels were stained as described (4). The pH of all buffers was measured at 25°C and 0.05 M, except for the electrophoresis buffers, which were measured at full strength. Buffers used for the autodigestion reaction were Na acetate (pH 5), Pipes·NaOH (pH 6 and 7), Tris·HCl (pH 7.4, 8, and 9), and glycine·NaOH (pH 10, 10.5, and 10.8).

Materials. Phenylmethanesulfonyl fluoride, isopropylthiogalactoside, trypsin, soybean trypsin inhibitor, Pipes, lysozyme, and ampicillin were obtained from Sigma; $NaDodSO_4$, hydroxyapatite, and Affi-Gel 501, from Bio-Rad; diisopropyl fluorophosphate, from Aldrich; AcA34, from LKB; calf thymus DNA, from Calbiochem; polyethyleneimine, from Miles; adenosine 5′-[γ-thio]triphosphate (ATP[S]), from P-L Biochemicals; phosphocellulose (P-11), from Whatman; and guanidine·HCl, from Bethesda Research Laboratories. Trypsin was dissolved and stored in 1 mM HCl.

RESULTS

The Autodigestion Reaction. lexA protein was purified from an overproducing strain to at least 99% of physical purity (Fig. 1, lanes 1–5). As previously shown (2, 4), highly purified protein was cleaved in a reaction requiring activated recA protein to yield two breakdown products (Fig. 1, lanes 6 and 7). When the incubation conditions were changed, however, cleavage no longer required recA protein. In a simple buffer consisting of 20 mM Tris·HCl, pH 9.0/10 mM $CaCl_2$, the protein broke down relatively rapidly with time into two stable products with the same mobilities as observed in the recA-catalyzed reaction (Fig. 1, lanes 8–12). We shall term this spontaneous reaction "autodigestion."

Properties of the Autodigestion Reaction. Standard conditions of 20 mM Tris·HCl, pH 9.0/10 mM $CaCl_2$ at 37°C were arbitrarily adopted for most of these studies. Variations in each of these parameters influenced the rate of the reaction. First, the reaction proceeded most efficiently at high pH values. At pH 7, 8, 9, and 10 in 10 mM $CaCl_2$, the half-life of the protein was about 24 hr, 4 hr, 45 min, and 10–15 min, respectively, and >>24 hr at pH 5 and 6. At pH 10.5 or 10.8, the rate was about the same as at pH 10, suggesting that an active group had been titrated. Second, the reaction proceeded more rapidly with increasing temperature to 50°C but far more slowly at 60°C, implying that a native conformation of the protein is needed for the reaction to proceed. The pH profile from 9 to 10.8 was about the same at 0, 19, 37, and 50°C. Third, the reaction occurred at a slow rate in the presence of EDTA; it was stimulated 2- to 3-fold by Mg^{2+} (10 mM), whereas Ca^{2+} (10 mM) and Co^{2+} (2 or 10 mM) gave about a 4-fold stimulation.

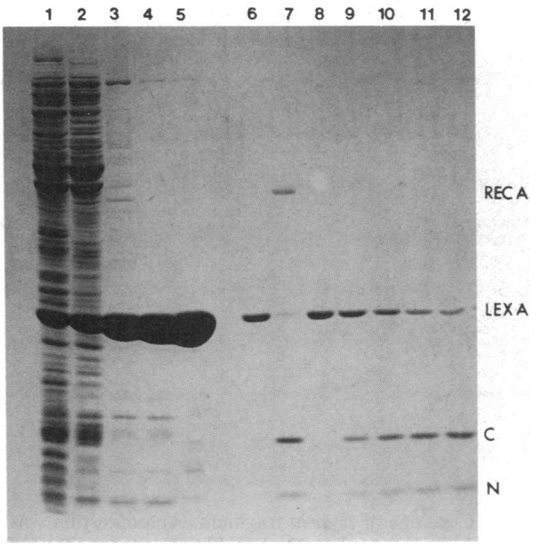

FIG. 1. Purification and specific cleavage of lexA protein. lexA protein was purified as described; aliquots of fractions I–V (lanes 1–5, respectively) were analyzed by electrophoresis (13% gel) and staining with Coomassie blue. Purified lexA protein was incubated under two conditions. (*i*) recA-dependent reaction. Samples (20 μl) containing lexA protein (100 μg/ml) were incubated for 60 min at 37°C in 20 mM Tris·HCl, pH 7.4/1 mM adenosine 5′-[γ-thio]triphosphate/2 mM $MgCl_2$/50 mM NaCl containing 0.67 μg of heat-denatured calf thymus DNA per ml in the absence (lane 6) or presence (lane 7) of purified recA protein (20 μg/ml), followed by addition of 10 μl of cracking buffer. (*ii*) Autodigestion reaction. lexA protein (100 μg/ml) was incubated at 37°C in 20 mM Tris·HCl, pH 9.0/10 mM $CaCl_2$; at 0 (lane 8), 30 (lane 9), 60 (lane 10), 90 (lane 11), or 120 min (lane 12), aliquots (20 μl) were removed and analyzed as above. "recA," "lexA," "C," and "N" denote the positions of recA protein, intact lexA protein, and the COOH-terminal and NH_2-terminal cleavage fragments, respectively.

Under standard conditions, the autodigestion reaction appeared to be intramolecular by two criteria. First, it followed roughly first-order kinetics, as judged by visual inspection of stained gels (such as in Fig. 1), implying that a given molecule has a constant probability of breaking down throughout the course of the reaction. Preliminary studies with radiolabeled protein confirmed that the reaction is first-order for at least one half-life, but the details of the kinetics at later times are unclear as yet. Second, the rate constant of the reaction appeared to be independent of protein concentration over the range 0.1–50 μM, as judged by staining with silver or Coomassie blue, and over the much wider range 20 pM–1 μM by studies with labeled protein and antibodies (not shown). Because lexA protein is a dimer at high concentrations (13) and has a dimerization constant of roughly 0.5–2 μM under conditions different from those used here (D. Burbee and J. Roberts, personal communication), the concentration independence of autodigestion suggests that a molecule of lexA protein can break down about equally well whether it is a monomer or part of a dimer. A similar conclusion was previously reached for the recA-catalyzed cleavage of lexA repressor (D. Burbee and J. Roberts, personal communication).

The optimal reaction conditions differed markedly from those used in the recA-catalyzed reaction. The nucleotide and polynucleotide cofactors known to be involved in the latter reaction were not added, and a divalent cation was not required. Moreover, when purified recA protein, its cofactors, or all three components were added under standard autodigestion conditions, the reaction was not stimulated except when high levels of recA protein were added with the cofactors (not shown).

Evidence Against a Contaminating Protease. The autodigestion reaction did not require recA protein. It was observed (Fig. 1) with a preparation of lexA protein isolated from a strain that carries a deletion of the *recA* gene; in addition, material isolated from a $recA^+$ strain and used for most of these studies contained <0.01% recA protein.

Although it is more difficult to rule out the presence of some other contaminating protease, several lines of evidence argue against this possibility. First, the reaction was seen in preparations of lexA protein that were purified to >99% homogeneity and showed 5–10 contaminating bands in overloaded gels at the 0.01–0.1% level (Fig. 1 and data not shown). Second, such a preparation, isolated from a $recA^+$ strain, was passed over an AcA34 sizing column in 6 M guanidine·HCl, and fractions containing lexA protein were dialyzed to refold the protein. The rate of breakdown across the protein peak was constant and was the same as that of the applied material (not shown). This result implies that, if the activity is due to a contaminant, the latter has the same mobility in the column as does lexA protein. Third, this same analysis was repeated, with the same result (not shown), on a partial tryptic digest of lexA protein that contained an active COOH-terminal fragment, TC1, roughly two-thirds the size of the intact protein (see below). This finding indicates that the mobility of the active species was changed upon mild trypsin treatment so that it ran with the TC1 fragment. Fourth, the kinetic properties of the reaction were consistent with its being intramolecular (see above) rather than intermolecular. Fifth, the reaction was observed with a preparation of lexA coded by a different overproducing plasmid and purified by a different procedure (4). A final weak line of evidence is that the activity was not inhibited by thiol antagonists, consistent with the absence of cysteine residues in lexA protein but inconsistent with the possibility of a contaminating thiol protease. Taken together, this evidence strongly suggests that the reaction is an intrinsic activity of lexA protein itself.

Specificity of the Autodigestion Reaction. The reaction was similar in its specificity to the recA-catalyzed reaction in two respects. First, cleavage occurred at the same $\overset{84}{\text{Ala}}$-$\overset{85}{\text{Gly}}$ bond in both reactions, as judged by NH_2-terminal amino acid sequence analysis of reaction mixtures. Second, a mutant form of the lexA protein, the lexA3 (Ind^-) protein, which is largely resistant to recA-catalyzed cleavage both *in vitro* and *in vivo* (3, 7), was similarly resistant to autodigestion ($t_{1/2}$ was 1–2 days at pH 10; data not shown). These data suggest that, despite the differences in reaction conditions (see above), the autodigestion reaction bears a close mechanistic relationship to the recA-catalyzed reaction (see also next section).

Autodigestion of λ Repressor. If the cleavage of lexA protein is an intrinsic activity of the protein, the role of recA protein in the recA-dependent cleavage reaction becomes unclear. If recA protein is not a protease (see *Discussion*), we would predict that other substrates for recA-promoted cleavage would also display autodigestion. To test this idea, phage λ repressor, the substrate with which the recA-catalyzed reaction was originally studied (5, 6), was incubated at pH 10. Like lexA protein, phage λ repressor broke down to two fragments of the same mobility as those given in the recA-dependent reaction (Fig. 2, lanes 1–6). This reaction was also stimulated by Ca^{2+} and by high pH (not shown) but not by addition of recA protein under these conditions (Fig. 2, lane 4). It proceeded at a rate far slower than that of autodigestion of lexA repressor. A comparable difference in rate for the two substrates has been observed *in vivo* and in the recA-catalyzed *in vitro* reaction (2, 4).

This parallelism with the recA-catalyzed reaction extends to a mutant form of the phage λ repressor, the Ind^s repres-

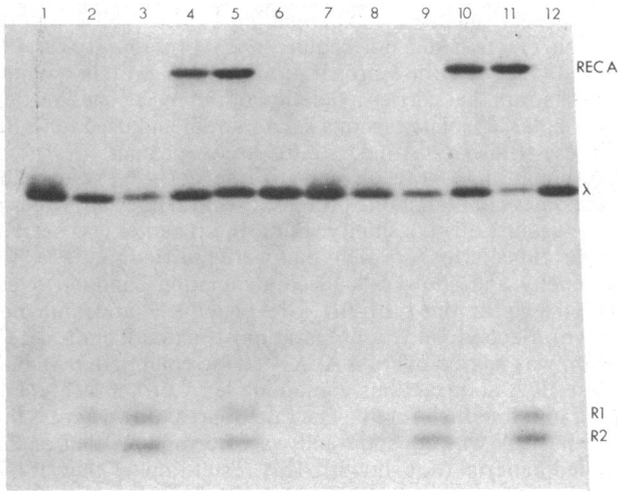

FIG. 2. Autodigestion of phage λ repressor. Autodigestion conditions (lanes 1–4, 7–10). Incubations were in 20 mM glycine·NaOH, pH 10.0/10 mM $CaCl_2$ containing 0.9 mM Tris·HCl (pH 8), 0.09 mM dithiothreitol, 0.45% glycerol, and 9 mM NaCl from the repressor storage buffer and 1 μM wild-type (lanes 1–4) or Ind^s (lanes 7–10) phage λ repressor. Incubation was for 0 (lane 1), 8 (lanes 2 and 4), or 24 hr (lane 3) for wild type and for 0 (lane 7), 4 (lanes 8 and 10), or 12 hr (lane 9) for Ind^s. recA protein (0.65 μM) was added to the samples in lanes 4 and 10. recA-dependent conditions. (lanes 5 and 6, 11 and 12). Incubation mixtures were as described in Fig. 1, except that the repressor preparations contributed the constituents listed above, recA protein was at 0.65 μM, and incubation was for 2 hr. Lanes: 5 and 6, wild-type λ repressor with and without recA protein, respectively; 11 and 12, Ind^s repressor with and without recA protein, respectively. In other autodigestion experiments (not shown), Ind^s repressor broke down 3–4 times more rapidly than did wild-type repressor. "recA," "λ," "R1," and "R2" denote recA protein, intact λ repressor, and the COOH- and NH_2-terminal fragments, respectively.

sor, which is more readily cleaved *in vivo* and in the recA-dependent *in vitro* reaction (14, 19). This form of the repressor also broke down more rapidly than did the wild-type repressor (Fig. 2 and data not shown) at the one concentration (1 μM) tested. The mutant protein is believed to be a better substrate than wild-type repressor because it dimerizes less readily (14, 19, D. Burbee and J. Roberts, personal communication) and because (in contrast to lexA protein) only monomers of phage λ repressor are susceptible to recA-catalyzed cleavage, so that a higher proportion of mutant protein is an efficient substrate for cleavage. The increased rate with which the Ind^s repressor undergoes autodigestion suggests, along with preliminary experiments at various concentrations of wild-type repressor (unpublished data), that only monomers of phage λ repressor efficiently break down in the autodigestion reaction as well.

Autodigestion of lexA is Catalyzed by the COOH-Terminal Two-Thirds of the Protein. To ask whether the entire lexA protein molecule was required for autodigestion or whether a portion would suffice, I took advantage of observations (unpublished data) that several proteases appear to attack lexA protein preferentially near its center, as previously found in the case of phage λ repressor (20). lexA protein was treated for a short period of time with trypsin, resulting in production of a relatively stable fragment, termed TC1, which comprised the portion of lexA protein from residue Leu-68 to the COOH-terminal residue Leu-202 and, therefore, included the $\overset{84}{\text{Ala}}$-$\overset{85}{\text{Gly}}$ cleavage site. This fragment was isolated, and, upon incubation under the standard conditions, it broke down into a fragment with the same mobility as the COOH-terminal fragment resulting from autodigestion of intact lexA protein (Fig. 3); presumably the short peptide from Leu-68 to Ala-84 also was produced but not detected in this analysis. Moreover, breakdown of the trypsin fragment proceeded at

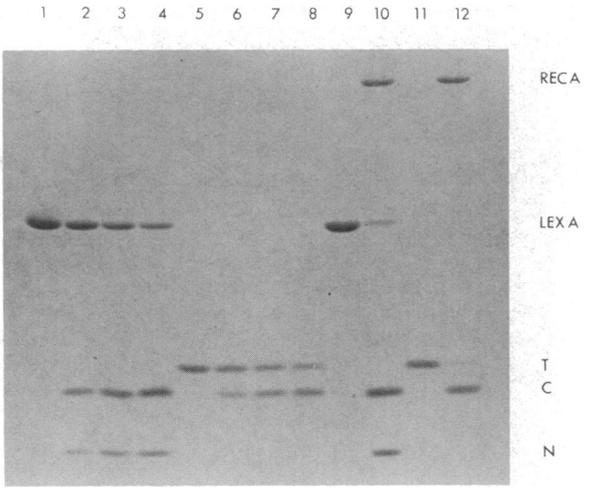

FIG. 3. Cleavage of trypsin fragment. Autodigestion was under the same conditions as described in Fig. 1, except that buffer D, diluted 1:20, was added with the protein; incubations contained intact lexA protein (2 μM, lanes 1–4) or fragment TC1 (about 1 μM, lanes 5–8) and were sampled at 0 (lanes 1 and 5), 30 (lanes 2 and 6), 60 (lanes 3 and 7), or 90 min (lanes 4 and 8). recA-dependent reaction conditions (lanes 9–12) were as in Fig. 1; samples contained intact lexA protein (lanes 9 and 10) or TC1 fragment (lanes 11 and 12) and were incubated in the presence (lanes 10 and 12) or absence (lanes 9 and 11) of recA protein (0.5 μM) for 1 hr. "recA," "lexA," "C," "N" are as in Fig. 1; "T" denotes fragment TC1.

about the same rate as did that of intact protein. These findings strongly imply that the active site for catalysis of the reaction lies within the COOH-terminal two-thirds of the protein and that it does not recognize determinants in the NH_2-terminal region.

recA protein also was able to degrade the COOH-terminal trypsin fragment under the recA-dependent reaction conditions (Fig. 3, lanes 11–12). This result, similar to that seen previously with an analogous fragment of phage λ repressor (21), indicates that the site recognized by recA protein also lies in this portion of lexA repressor.

DISCUSSION

These findings indicate that lexA protein contains an intrinsic protease activity making it capable of cleaving itself. The reaction appears to be intramolecular, a type of protease activity seen to date only in the case of pepsinogen (22) and analogous to the self-splicing RNA molecules recently described (23).

The autodigestion reaction shows the same site and substrate specificity as does the recA-dependent cleavage reaction. This pattern suggests that the recA-catalyzed and spontaneous reactions follow, at least in part, a similar reaction pathway. It also suggests that recA protein may play an indirect role in cleavage by stimulating the autodigestion reaction. recA could do this by lowering the pK of a side chain in the repressors or by acting as an allosteric effector. Alternatively, recA protein may yet prove to be a protease, cleaving at a bond already made labile by the structure of the substrate.

The data give little clue as to the detailed chemical mechanism of the autodigestion reaction, beyond the strong implication that it is intramolecular and that it probably proceeds about equally well in the monomer and dimer forms of the protein. Preliminary inhibitor studies have not been informative. The reaction might proceed by hydroxide ion or general base catalysis or by way of a covalent intermediate between the carbonyl group of the cleavage site and a nucleophilic aminoacyl side chain (as with chymotrypsin). The stimulation at alkaline pH does not distinguish between these possibilities but is clearly of mechanistic importance.

The data show that lexA protein is more complex than pre-

viously supposed. A variety of evidence (ref. 1 and unpublished data) has suggested that its NH_2-terminal portion contains the specific DNA-binding site as in the case of phage λ repressor. The present findings suggest that the COOH-terminal two-thirds of lexA protein contains three conceptually separable sites: first, the cleavage site—analogous to the substrate in an enzyme–substrate reaction; second, the active site—the side chains that make the Ala-Gly bond labile and perhaps those that attack and hydrolyze it; and finally, the recA-recognition site, where recA protein binds to play its role in the recA-dependent reaction.

The fact that λ repressor also shows the autodigestion reaction argues that it has these same functions, and other data (21) have shown that the recA-recognition site lies in the COOH-terminal two-thirds of phage λ repressor. The COOH-terminal portions of lexA and phage λ repressors share considerable homology (24), mostly in several clusters, suggesting the possibility that an enzymatically active site has been conserved. The central portions of the two proteins show limited homology around the cleavage site, followed by a long tract of nonhomology towards the COOH terminus. Mutants in the phage λ *cI* repressor gene are known that map in its COOH-terminal portion and that block prophage induction, presumably by rendering the protein insensitive to cleavage *in vivo* (25, 26); however, in this case the mutants might affect dimerization and, hence, the availability of active substrate for *in vivo* cleavage (see 1, 14, 19).

Previous *in vivo* evidence (7) has shown that the stability of lexA repressor varies greatly during the SOS regulatory cycle. Because the protein breaks down to the same cleavage products as those produced *in vitro*, these data were interpreted to mean that variations in lexA stability directly reflected variations in the level of activated recA protein. Because the present data imply that the reaction pathway for *in vivo* cleavage deeply involves the chemistry of lexA protein, it becomes pertinent to ask whether other molecules than recA protein might also modulate the *in vivo* stability of lexA protein by affecting its reactivity. In this case, variations in the level of such modulators might also play a role in controlling the state of the SOS regulatory system (see also ref. 27).

Aside from the possible complication just mentioned, the new data do not significantly alter our view of the SOS regulatory system, in the sense that the regulatory circuitry should still operate by means of the same pathways and interactions as previously believed. It still seems clear that recA function is required *in vivo* for cleavage to occur, as judged both directly by antibody techniques (7, 28) and indirectly by the fact that *recA*$^-$ mutants block derepression of the SOS system and prophage induction (see ref. 1). If we change the nomenclature from "recA protease" to "the activated form of recA protein" or "activated recA", the description of the circuitry in the SOS system is the same as before.

This is an interesting example of viewing a system at several levels: at the higher level—that of the regulatory system—the important point is that the constituents interact to produce the needed effect, and the details of the interaction, which we study at a lower level of organization, do not matter greatly as long as the mechanism works. Conversely, understanding the system at the lower, more detailed level helps us to attain a wider view of cellular metabolism.

For instance, there is a variety of evidence that the activated form of recA protein plays other regulatory and metabolic roles besides controlling the SOS system. Perhaps activated recA can cause allosteric changes in other proteins in addition to the repressors. The range of possible mechanisms for its action would then be wide indeed. For example, inactivation of φ80 repressor (whose cleavage has not yet been reported; see ref. 1) might take place by forming a tight complex with activated recA, titrating the repressor off its operators, and the general pattern of gene expression after prophage induction could proceed in much the same way as with phage λ and its cleavable repressor.

An especially intriguing process requiring activated recA protein is induced mutagenesis (ref. 29; D. Mount, D. Ennis, B. Fisher & S. Edmiston, personal communication). Perhaps, again, activated recA protein might interact with other protein, such as components of the replication fork, to have its mutagenic effect, rather than by the specific cleavages of such proteins as has been suggested in the past. The present data suggest that a search for other cleavage reactions might be futile and that further study should focus on the interaction of other cellular proteins with activated recA protein.

I am grateful to R. L. Baldwin, Don Ennis, William Jencks, Pamela Keim, David Mount, John Rupley, Michael Wells, Kenneth Wertman, and Howard White for helpful discussions; to David Burbee, Jeffrey Roberts, and David Mount for communicating unpublished results; to Jürgen Brosius and David Mount for plasmid and bacterial strains; to Sylvia Hill and Celeste Yanisch-Perron for excellent technical assistance; to Sara Cohen and Steve West for proteins; to Alan Smith for amino acid sequence analysis; and to Gail Hewlett for help with the figures. This work was supported by National Institutes of Health Grant GM24178.

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